of boosting vaccine-coverage rates. Requiring HPV vaccination by law will almost certainly achieve more widespread protection against the disease than will policies that rely exclusively on persuasion and education. In the view of advocates, this effectiveness provides a clear justification. "The only way to ensure that as many girls as possible receive the HPV vaccine is to require it before they enter middle school," said Beverly Hammerstrom, the Michigan state senator who introduced the legislation. Whether such a mandate might extend to boys, should the product be approved for such use, remains uncertain.

A critical question is whether achieving a higher level of coverage justifies the infringement on parental autonomy that compulsory vaccination inevitably entails. Different ethical frameworks that accord varying weights to communitarian and individualistic values will lead to contrasting answers to this question.

Ethical and epidemiologic analyses are essential to decisions about mandating the HPV vaccine; so are political calculations. Any new vaccine that a state adds to its list of requirements must be judged in the context of both the increasingly lengthy and complex regimen of vaccines that children now receive and the possibility that additional mandates may inflame grassroots opposition, be it religious, philosophical, or ideological.[5] Although issues of religion and adolescent sexuality have dominated the discussion, the move to require HPV vaccination raises broad questions about the acceptability of mandatory public health measures, the scope of parental autonomy, and the role of political advocacy in determining how preventive health measures are implemented.

Dr. Colgrove is an associate research scientist at the Center for the History and Ethics of Public Health, Department of Sociomedical Sciences, Mailman School of Public Health, Columbia University, New York.

1. Salmon DA, Teret SP, MacIntyre RC, Salisbury D, Burgess MA, Halsey NA. Compulsory vaccination and conscientious or philosophical exemptions: past, present, and future. Lancet 2006;367:436-42.
2. Colgrove JK. State of immunity: the politics of vaccination in twentieth-century America. Berkeley: University of California Press, 2006.
3. Zimmerman RK. Ethical analysis of HPV vaccine policy options. Vaccine 2006;24:4812-20.
4. Lo B. HPV vaccine and adolescents' sexual activity. BMJ 2006;332:1106-7.
5. Temte JL. Should all children be immunised against hepatitis A? BMJ 2006;332:715-8.

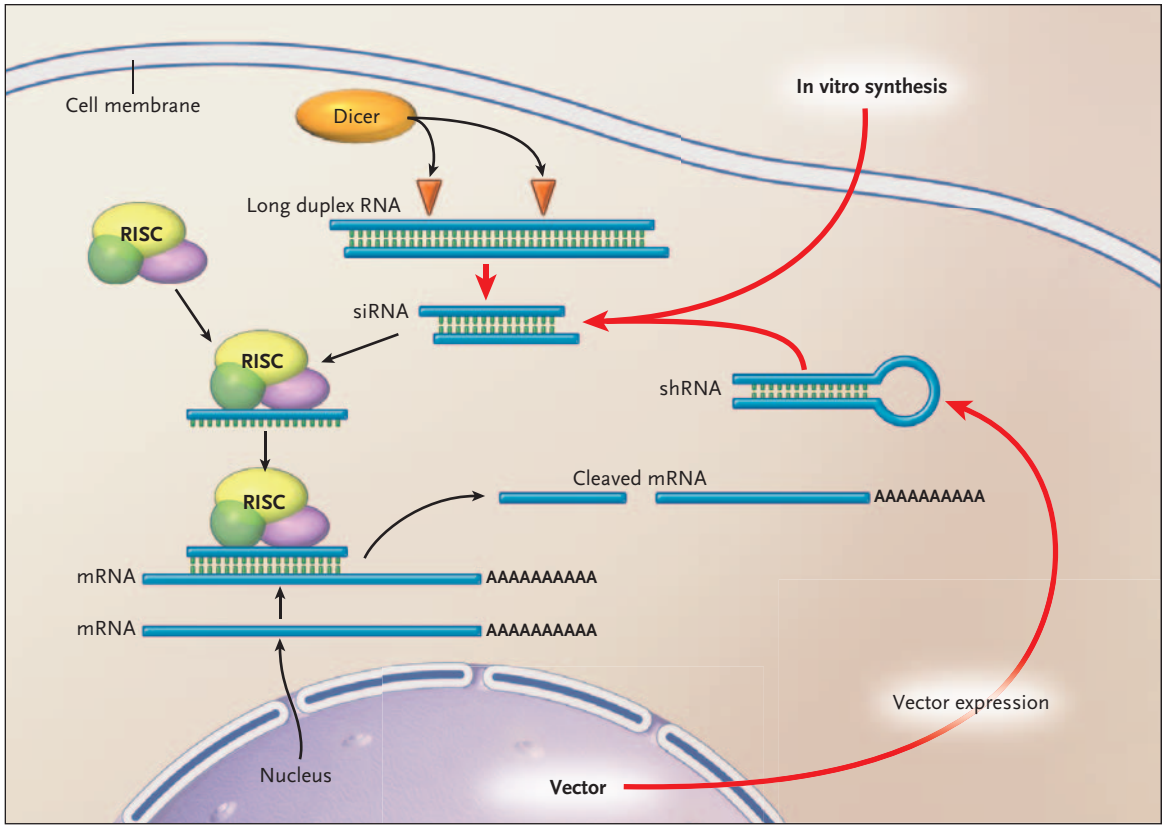
Exploring the Uses of RNAi — Gene Knockdown and the Nobel Prize

René Bernards, Ph.D.

The Nobel Prize in Physiology or Medicine was awarded this year to Andrew Fire (Stanford University School of Medicine) and Craig Mello (University of Massachusetts Medical School) for their discovery of a new form of gene silencing. Nearly 9 years ago, Fire and Mello and their colleagues reported that exposing cells of the nematode *Caenorhabditis elegans* to double-stranded RNA resulted in specific and efficient gene silencing.[1] They also observed that double-stranded RNA is far more potent than sense or antisense RNA in silencing the gene that shares its sequence, and they dubbed the silencing process "RNA interference" (RNAi). Because RNAi rarely leads to the complete abrogation of gene expression, its effect is often described as a "knockdown" of gene expression. At first glance, RNAi seems similar to the antisense approach to gene silencing, but it is far more effective and has a different mechanism.

In plants and nematodes, the introduction of long double-stranded RNA into a cell leads to its cleavage into shorter fragments. These fragments are powerful silencers of gene expression and are therefore called "small interfering RNA" (siRNA). They are recruited into a protein complex that positions the antisense strand so that it acts as a snare for the RNA transcript to which it is complementary. Once bound to this snare, the RNA transcript is cleaved by the complex and is degraded (see diagram). In lower organisms, RNAi is thought to function as a primitive immune system, protecting against viruses (which often generate double-stranded RNA as replication intermediates) and transposable elements (also known as "jumping genes").

In most mammalian cells, long double-stranded RNA provokes an interferon response as part of an antiviral defense. This interferon response induces a global shutdown of protein synthesis, thus precluding the use of long double-stranded RNA for specific gene silencing. This obstacle can be overcome by using short double-stranded RNA (less than 30 base pairs in length), which evades the radar of the mammalian interfer-



The Mechanism of RNA Interference.

Long double-stranded RNA is cleaved by a nuclease (Dicer) into small interfering RNA (siRNA; orange triangles indicate cleavage sites). The siRNA can also be synthesized in vitro and introduced into cells (which allows for only transient inhibition of gene expression) or can be produced in the cells by DNA-based vector systems encoding short hairpin RNA (shRNA), which allows for persistent gene silencing. (Red arrows indicate these different origins of siRNA.) siRNA is incorporated into the RNA-induced silencing complex (RISC), which exposes the antisense strand of the siRNA molecule to the cellular milieu, permitting it to recognize messenger RNA (mRNA) molecules that contain a perfect complementary sequence. Once bound to the RISC, the mRNA molecules are cleaved and degraded. The corresponding gene is thus silenced through a posttranscriptional mechanism.

on response and effects strong and specific gene silencing. Moreover, simple expression vectors that direct the synthesis of so-called short hairpin RNA (shRNA) — which the cell converts into siRNA — can also mediate gene silencing in mammalian cells. Integration of such vectors into the host genome results in a continuous supply of shRNA and, thus, persistent silencing. RNAi has very quickly become the basis of a booming business, with many vendors offering siRNA sets or shRNA vector collection kits that target nearly all the genes in the human and mouse genomes.

RNAi has many applications in biomedical research, including drug development. A simple and useful application is the validation of specific genes that are presumed to function in a process of interest: researchers can knock down the expression of the gene (in cell culture or animal model) and observe the consequences. Because RNAi allows for the rapid and efficient suppression of the expression of any protein in almost any type of cell, its use can expedite the evaluation of candidate targets for drug development. A variation on this theme is the use of RNAi to systematically screen large sets of genes for their involvement in specific processes. This approach is particularly powerful if members of "druggable" gene families (such as kinases, ion channels, or G protein–coupled receptors) are targeted, because a "hit" in such a screening represents a starting point for the development of a drug.

An exciting new concept in the development of cancer drugs is the genotype-specific drug target — the protein whose inactivation is toxic only to cells carrying a defined (cancer-specific) genetic lesion. In theory, drugs targeting the products of such genes would

Family Chemistry — A Nobel Tradition

This year's Nobel Prize in Chemistry is being awarded to Roger Kornberg of the Stanford University School of Medicine for delineating the way in which DNA is transcribed into RNA. The process of transcription is mediated by many molecules. The centerpiece, however, is the enzyme RNA polymerase II. Using the same technique that Watson and Crick used to fathom the structure of DNA, Kornberg showed that polymerase II binds DNA and provides a restrictive scaffold that permits the addition (as the polymerase moves down the DNA molecule) of only the nucleotide that is complementary to the DNA base engaged by the polymerase. This mechanism ensures faithful transcription of DNA into RNA, which is central to the health of the cell and the organism of which it is part. Kornberg's crowning achievement, however, was not his detailed characterization — down to the atom — of molecular components of the transcriptional machinery, but a synthesis of the parts into a four-dimensional model of the process.

If you have a sense of déjà vu, it may be explained by the fact that Kornberg's father, Arthur Kornberg, shared the Nobel Prize in Physiology or Medicine in 1959 for figuring out how DNA replicates. In this sense, the Kornbergs follow a tradition. There are six other parent–child pairs of Nobel laureates — seven if you count Irène Curie twice: her parents shared one Nobel Prize, and her mother won a second on her own.

Elizabeth G. Phimister, Ph.D.

be more selective for cancer cells than are the current generation of broadly acting cytotoxic drugs, since they would require the presence of a cancer-specific lesion to exert their cytotoxic effects. RNAi technology is exquisitely suited to uncovering synthetic lethal interactions — a combination of two nonlethal events that together result in cell death — in mammalian cells; indeed, the first RNAi screenings to uncover such genetic interactions were recently described.[2,3] The heterogeneity of tumors, however, may represent an obstacle to translating this approach into a useful clinical tool.

Resistance to therapy often stymies the treatment of disease, and mechanisms of resistance are usually obscure. RNAi can be used to identify genes involved in drug resistance: one could knock down genes in drug-sensitive cells in vitro, expose the cells to the drug, and then see which cells survive — and hence, which genes are necessary for drug sensitivity. The relevance to the drug response of these genes and the pathways in which they lie could then be validated in clinical trials.

Many diseases are caused by the inappropriate activity of specific genes, and the selective silencing of such genes through RNAi represents a potential therapeutic strategy for such diseases. As compared with small-molecule drugs, siRNA is versatile in that it can target any gene for suppression, and it is not subject to the costly and time-consuming process of small-molecule drug development, a process that fails more often than it succeeds. However, the road to successful therapeutic application of siRNA is likely to be treacherous, and those who attempt to travel it will encounter at least three obstacles.[4]

First, although chemical modification has been shown to improve the stability of siRNA, further improvement is required for its systemic delivery in vivo. Chemical modification may also be used to target the siRNA to specific cell types. In the short term, at least, delivery of siRNA to confined compartments (such as the eye) seems promising because it bypasses many of the problems associated with systemic delivery. A clinical trial involving the intraocular injection of siRNA to treat age-related macular degeneration is ongoing. Second, the problem of unpredictable "off-target" effects (the silencing of genes other than the intended transcript) must be addressed. Finally, the question of potential toxic effects must be laid to rest. Nevertheless, the successful application of RNAi to a broad range of animal models of disease — for diseases such as amyotrophic lateral sclerosis, spinocerebellar ataxia, and atherosclerosis and infections caused by the respiratory syncytial virus, parainfluenza virus, herpes simplex virus 2, and the hepatitis B and C viruses — augurs well.

It is unusual for the Nobel Committee to award a prize in medicine so soon after the relevant discovery. But then, hardly ever has such a discovery given rise so quickly to such a broad range of promising medical applications.

Dr. Bernards is the head of the Division of Molecular Carcinogenesis at the Netherlands Cancer Institute, Amsterdam.

1. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 1998; 391:806-11.
2. Ngo VN, Davis RE, Lamy L, et al. A loss-of-function RNA interference screen for molecular targets in cancer. Nature 2006;441:106-10.
3. Rottmann S, Wang Y, Nasoff M, Deveraux QL, Quon KCA. A TRAIL receptor-dependent synthetic lethal relationship between MYC activation and GSK3beta/FBW7 loss of function. Proc Natl Acad Sci U S A 2005;102:15195-200.
4. Morris KV. Therapeutic potential of siRNA-mediated transcriptional gene silencing. Biotechniques 2006;Suppl:7-13.